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A Novel Cytochrome P450 Expressed Primarily in Brain*

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hct-1 **(hippocampal transcript) was detected in a differential screen of a rat hippocampal cDNA library. Expression of *hct-1* was enriched in the formation but was also detected in rat liver and kidney, though at much lower levels; expression was barely detectable in testis, ovary, and adrenal. In liver, unlike brain, expression was sexually dimorphic; hepatic expression was greatly reduced in female rats. In mouse, brain expression was widespread, with the highest levels being detected in corpus callosum; only low levels were detected in liver. Sequence analysis of rat and mouse *hct-1* cDNAs revealed extensive homologies with cytochrome P450s (CYPs), a diverse family of heme-binding monooxygenases that metabolize a range of substrates including steroids, fatty acids, and xenobiotics. Among the CYPs, *hct-1* is most similar (39% at the amino acid sequence) to cholesterol 7α-hydroxylase (CYP7) and contains a postulated steroidogenic domain present in other steroid-metabolizing CYPs but clearly represents a type of CYP not previously reported. Genomic Southern analysis suggests that a single gene corresponding to *hct-1* is present in mouse, rat, and human. *hct-1* is unusual in that, unlike all other CYPs described, the primary site of expression is in the brain. Similarity to CYP7 and other steroid-metabolizing CYPs may argue that *hct-1* (CYP7B) plays a role in steroid metabolism in brain, notable because of the documented ability of brain-derived steroids (neurosteroids) to modulate cognitive function *in vivo*.**

Cytochromes P450, a diverse group of heme-containing monooxygenases (termed CYPs)[1] (for nomenclature see Nelson *et al.* (1)), catalyze a variety of oxidative conversions, notably of steroids but also of fatty acids and xenobiotics. Though most abundantly expressed in the testis, ovary, placenta, adrenal, and liver, the brain is a further site of CYP expression (2–6). Several CYP activities or mRNAs have been reported in the nervous system, predominantly of types metabolizing fatty acids and xenobiotics (subclasses CYP2C, -2D, -2E, and -4 (6, 7)). However, primary rat brain-derived glial cells can synthesize pregnenolone and progesterone *in vitro* (8). Mellon and Deschepper (9) provided molecular evidence for the presence, in brain, of key steroidogenic enzymes CYP11A1 (scc) and CYP11B1 (11β) but failed to detect CYP17 (c17) or CYP11B2 (AS). Though CYP21A1 (c21) activity is reported to be present in brain (10) authentic CYP21A1 transcripts were not detected (11).

Interest in brain steroid metabolism has been fueled by the finding that adrenal- and brain-derived steroids (neurosteroids) can modulate cognitive function and synaptic plasticity (reviewed in Refs. 12–17). For instance, pregnenolone and steroids derived from it are reported to have memory-enhancing effects in mice (18, 19). However, the full spectrum of brain CYPs and the biological roles of their metabolites *in vivo* have not been established.

To investigate such regulation of brain function our studies have focused on the hippocampus, a brain region important in learning and memory. Patients with lesions that include the hippocampus display pronounced deficits in the acquisition of new explicit memories; in rat, neurotoxic lesions to the hippocampus lead to a pronounced inability to learn a spatial navigation task, such as the water maze (20). Hippocampal synapses, notably those in region CA1, display a particularly robust form of activity-dependent plasticity known as long term potentiation (LTP) (21) that satisfies some of the requirements for a molecular mechanism underlying memory processes, persistence, synapse specificity, and associativity. LTP is thought to be initiated by calcium influx through the NMDA (*N*-methyl-D-aspartate) subclass of receptor activated by the excitatory neurotransmitter, L-glutamate (reviewed in Ref. 22); occlusion of NMDA receptors with AP5 both blocks LTP and the acquisition of the spatial navigation task (23). *In vivo*, simultaneous release of γ-aminobutyric acid from inhibitory interneurons inhibits NMDA channel opening and LTP induction (22). It is of note that some naturally occurring steroids, such as pregnenolone sulfate, act as agonists of the γ-aminobutyric acid receptor (*e.g.* see Refs. 24 and 25) and may also directly modulate NMDA currents (26, 27). Though brain steroids principally appear to exert their effects via the γ-aminobutyric acid and NMDA receptors, there are indications that neurosteroids may also interact with σ and progesterone receptors (28, 29). However, the pathways of CYP-mediated steroid metabolism in the central nervous system have not been fully elucidated.

In addition, non-steroid CYP metabolites also play important roles in brain; CYP-mediated metabolism of psychoactive agents (30) and CYP metabolites of arachidonic acid such as prostanoids and eicosanoids (31) clearly contribute to the reg-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U36992 and U36993.

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[1] The abbreviations used are: CYP, cytochrome P450 (for simplicity no systematic distinction is made between genes encoding mouse (*Cyp*), rat, and human (*CYP*) orthologs or their protein products); LTP, long term potentiation; NMDA, *N*-methyl-D-aspartate; kb, kilobase(s); nt, nucleotide(s).

ulation of brain function.

As part of a study into the molecular biology of the hippocampal formation and the mechanisms underlying synaptic plasticity, we have sought molecular clones corresponding to mRNA's expressed selectively in the formation. One such cDNA, *hct-1* (for hippocampal transcript), was isolated from a cDNA library prepared from adult rat hippocampus. Sequence analysis revealed that *hct-1* is a novel cytochrome P450 most closely related to cholesterol- and steroid-metabolizing CYPs but, unlike other CYPs, is predominantly expressed in brain. We present molecular characterization of *hct-1* coding sequences from rat and mouse and their expression patterns and discuss the possible role of *hct-1* in the central nervous system.

MATERIALS AND METHODS

Preparation of cDNA Libraries—Following anesthesia (sodium pentobarbital) of adult rats (Lister hooded) the hippocampal formation was dissected, including areas CA1–3 and dentate gyrus, subiculum, alvear, and fimbrial fibers but excluding fornix and afferent structures such as septum and entorhinal cortex. The remainder of the brain was also pooled taking care to exclude hippocampal tissue. Total RNAs were prepared by a standard guanidinium isothiocyanate procedure, centrifugation through a CsCl cushion, and poly(A)$^+$ mRNA selected by affinity chromatography on oligo(dT)-cellulose. First strand cDNA synthesis used a *NotI* adaptor primer (5′-dCAATTCGCGGCCGC(T)15–3′) and Moloney murine leukemia virus reverse transcriptase; second strand synthesis was performed by RNase H treatment, DNA polymerase I fill-in, and ligase treatment. Following the addition of hemiphosphorylated *EcoRI* adaptors (5′-dCGACAGCAACGG-3′ and 5′-dAATTCCGTTGCTGTCG-3′) and cleavage with *NotI* the cDNA was inserted between the *NotI* and *EcoRI* sites of bacteriophage λ vector λ-ZAPII (Stratagene). The mouse liver cDNA library, also established as *NotI-EcoRI* fragments in a λ-gt10 vector, was a kind gift of B. Luckow and K. Kästner, Heidelberg.

Differential Hybridization Screening—Recombinant bacteriophage plaques were transferred in duplicate to Hybond-N membranes (Amersham Corp.), denatured (0.5 M NaOH, 1.5 M NaCl, 4 min), renatured (1 M Tris-HCl pH 7.4, 1.5 M NaCl), rinsed, dried, and baked (2 h, 80 °C). Hybridization as described (32) used a radiolabeled probe prepared by Moloney murine leukemia virus reverse transcriptase copying of poly(A)$^+$ RNA (from either hippocampus or the remainder of brain) into cDNA in the presence of [α-^{32}P]dCTP and unlabeled dGTP, dATP, and dTTP according to standard procedures. Following washing and exposure for autoradiography, differentially hybridizing plaques were repurified. Inserts were transferred to a pBluescript vector either by cleavage and ligation or by using *in vivo* excision using the ExAssist/SOLR system (Stratagene).

Northern Analysis—Total RNA was extracted by tissue homogenization in guanidinium thiocyanate according to a standard procedure and further purified by centrifugation through a CsCl cushion. Where appropriate, poly(A)$^+$ RNA was selected on oligo(dT)-cellulose. Electrophoresis of RNA (10 μg) on 1% agarose in the presence of 7% formaldehyde was followed by capillary transfer to nylon membranes, baking (2 h, 80 °C), and rinsing in hybridization buffer (0.25 M sodium phosphate, pH 7.2; 1 mM EDTA, 7% SDS, 1% bovine serum albumin) as described (32). Probes were prepared by random priming of DNA polymerase copying of denatured double-stranded DNA. Hybridization (16 h, 68 °C) was followed by washing (3 times, 20 mM sodium phosphate, pH 7.2, 1 mM EDTA, 1% SDS, 20 min, 68 °C), and membranes were exposed for autoradiography. The loading control probe was a 0.5-kb cDNA encoding the ubiquitously expressed rat ribosomal protein S26 (33).[2]

In Situ Hybridization—Synthetic *hct-1* oligonucleotide probes 5′-dGACAGGTTTTGTGACCCAAAACAAACTGGATGGATCGCAATC-3′ (rat, 55% G + C) and 5′-ATCACGGAGCTCAGCACATGCAGCCTTACTCTGCAAAGCTTC-3′ (mouse, 48% G + C) were labeled using terminal transferase (Boehringer Mannheim) and α-^{35}S-dATP (Amersham) according to the manufacturer's instructions. The control probe, 5′-dAGCCTTCTGGGTCGTAGCTGACTCCTGCTGCTGAGCTGCAACAGCTTT-3′ (56% G + C) was based on human opsin cDNA (34). Frozen coronal 10-μm sections of brain were fixed (4% paraformaldehyde, 10 min), rinsed, treated with proteinase K (20 μg/ml in 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 5 min), rinsed, and refixed with paraformaldehyde as before. Following acetylation (0.25% acetic anhydride, 10 min) and rinsing, sections were dehydrated by passing though increasing ethanol concentrations (30, 50, 70, 85, 95, 100, and 100%, each for 1 min except the 70% step (5 min)). Following $CHCl_3$ treatment (5 min) and rinsing in ethanol, sections were dried before hybridization. Hybridization in buffer (4 × standard saline citrate (1 × SSC = 0.15 M NaCl, 0.015 M Na_3citrate), 50% v/v formamide, 10% w/v dextran sulfate, 1 × Denhardt's solution, 0.1% SDS, 500 μg/ml denatured salmon sperm DNA, 250 μg/ml yeast tRNA) was for 16 h at 37 °C. Slides were washed (4 × 15 min, 1 × SSC, 60 °C; 2 × 30 min, 1 × SSC, 20 °C), dipped into photographic liquid emulsion (LM-1, Amersham Corp.), exposed, and developed according to the manufacturer's specifications. Slides were counterstained with 1% methyl green.

DNA Sequence Characterization—Dideoxy sequencing of cDNAs in pBluescript II KS and SK vectors was performed using the Sequenase 2.0 kit (Amersham Corp.) according to the manufacturer's instructions. To sequence larger cDNAs sequential exonuclease III deletions were produced (Erase-a-base, Promega Biotech). Sequence data, obtained for both strands of the larger *hct-1* cDNAs, were analyzed using the University of Wisconsin Genetics Computer Group package (UWGCG Version 7, 1991).

Southern Hybridization—Genomic DNA prepared from mouse or rat liver or from human lymphocytes was digested with the appropriate restriction endonuclease, resolved by agarose gel electrophoresis (0.7%), and transferred to Hybond-N membranes. Following baking (2 h, 80 °C), hybridization conditions were as described for Northern analysis.

RESULTS

Differential Screening of a Rat Hippocampus cDNA Library—To identify genes whose expression is enriched in the hippocampal formation we performed a differential hybridization screen of a hippocampal cDNA library. Adult rat hippocampal RNA was converted to double-stranded cDNA and inserted as *EcoRI-NotI* fragments into a bacteriophage λ vector. Duplicate lifts from 500,000 plaques were screened with radiolabeled cDNA probes prepared from either hippocampus or "rest of brain." Some 360 clones gave a substantially stronger hybridization signal with the hippocampus probe than with the rest of brain probe; 49 were analyzed in more depth. *In vivo* excision was used to transfer the inserts to a plasmid vector for partial DNA sequence studies. Of these, 21 were novel (not presented here); others were known genes whose expression is enriched in hippocampus but not specific to the formation (*e.g.* the rat amyloidogenic protein (35)). Northern analysis was first performed using radiolabeled probes corresponding to the 21 novel sequences. While three (12.10a, 14.5a, and 15.13a) identified transcripts specific to the hippocampus, 12.10a and 15.13a both hybridized to additional transcripts whose expression was not restricted to the formation (not presented). Clone 14.5a appeared to identify transcripts enriched in hippocampus (see below) and was dubbed *hct-1*.

Rat hct-1 Encodes a Cytochrome P450—The insert of clone 14.5a (300 nt) was used to rescreen the hippocampal cDNA library. Four positives were identified (clones 14.5a-5, -7, -12, and -13), and the region adjacent to the poly(A) tail was analyzed by DNA sequencing. While clones 5 (0.7 kb) and 12 (1.4 kb) had the same 3′ end as the parental clone, clone 7 (0.9 kb) had a different 3′ end consistent with utilization of an alternative polyadenylation site (see below). Clone 13 (2.5 kb), dubbed *hct-2*, appeared unrelated to *hct-1* (not presented).

Clones 12 and 7 were then fully sequenced and compared with the data base. Homology was detected between clone 12 and the human (36) and rat (37, 38) cDNA's encoding cholesterol 7α-hydroxylase (CYP7), though the sequences are clearly distinct. At the nucleic acid level, the 1428-nt cDNA clone for rat *hct-1* shared 55% identity over an 1100-nt overlap with human CYP7 and 54% identity over a 1117-nt overlap with rat CYP7 (not presented). Fig. 1 gives the partial cDNA sequences of rat *hct-1* and the encoded polypeptide.

hct-1 mRNA Expression in Rat—Rat *hct-1* clone 14.5a/12 (1.4

[2] M. Richardson, unpublished data.

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A  L  E  Y  Q  Y  V  M  K  N  P  K  Q  L  S  F  E  K  F  S
GCCTTGGAGTACCAGTATGTAATGAAAAACCCAAAACAATTAAGCTTTGAGAAGTTCAGC   60
R  R  L  S  A  K  A  F  S  V  K  K  L  L  T  N  D  D  L  S
CGAAGATTATCAGCGAAAGCCTTCTCTGTCAAGAAGCTGCTAACTAATGACGACCTTAGC  120
N  D  I  H  R  G  Y  L  L  L  Q  G  K  S  L  D  G  L  L  E
AATGACATTCACAGAGGCTATCTTCTTTTACAAGGCAAATCTCTGGATGGTCTTCTGGAA  180
T  M  I  Q  E  V  K  E  I  F  E  S  R  L  L  K  L  T  D  W
ACCATGATCCAAGAAGTAAAAGAAATATTTGAGTCCAGACTGCTAAAACTCACAGATTGG  240
N  T  A  R  V  P  D  F  C  S  S  L  V  P  E  I  T  F  T  T
AATACAGCAAGAGTATTTGATTTCTGTAGTTCACTGGTATTTGAAATCACATTTACAACT  300
I  Y  G  K  I  L  A  A  N  K  K  Q  I  I  S  E  L  R  D  D
ATATATGGAAAAATTCTTGCTGCTAACAAAAAACAAATTATCAGTGAGCTGAGGGATGAT  360
F  L  K  F  D  D  H  F  P  Y  L  V  S  D  I  P  I  Q  L  L
TTTTTAAAATTTGATGACCATTTCCCATACTTAGTATCTGACATACCTATTCAGCTTCTA  420
R  N  A  E  F  M  Q  K  K  I  I  K  C  L  T  P  E  K  V  A
AGAAATGCAGAATTTATGCAGAAGAAAATTATAAAATGTCTCACACCAGAAAAAGTAGCT  480
Q  M  Q  R  R  S  E  I  V  Q  E  R  Q  E  M  L  K  K  Y  Y
CAGATGCAAAGACGGTCAGAAATTGTTCAGGAGAGGCAGGAGATGCTGAAAAAATACTAC  560
G  H  E  E  F  E  I  G  A  H  H  L  G  L  L  W  A  S  L  A
GGGCATGAAGAGTTTGAAATAGGAGCACATCATCTTGGCTTGCTCTGGGCCTCTCTAGCA  600
N  T  I  P  A  M  F  W  A  M  Y  Y  L  L  Q  H  P  E  A  M
AACACCATTCCAGCTATGTTCTGGGCAATGTATTATCTTCTTCAGCATCCAGAAGCTATG  660
E  V  L  R  D  E  I  D  S  F  L  Q  S  T  G  Q  K  K  G  P
GAAGTCCTGCGTGACGAAATTGACAGCTTCCTGCAGTCAACAGGTCAAAAGAAAGGACCT  720
G  I  S  V  H  F  T  R  E  Q  L  D  S  L  V  C  L  E  S  A
GGAATTTCTGTCCACTTCACCAGAGAACAATTGGACAGCTTGGTCTGCCTGGAAAGCGCT  780
I  L  E  V  L  R  L  C  S  Y  S  S  I  I  R  E  V  Q  E  D
ATTCTTGAGGTTCTGAGGTTGTGCTCCTACTCCAGCATCATCCGTGAAGTGCAAGAGGAT  840
M  D  F  S  S  E  S  R  S  Y  R  L  R  K  G  D  F  V  A  V
ATGGATTTCAGCTCAGAGAGTAGGAGCTACCGTCTGCGGAAAGGAGACTTTGTAGCTGTC  900
F  P  P  M  I  H  N  D  P  E  V  F  D  A  P  K  D  F  R  P
TTTCCTCCAATGATACACAATGACCCAGAAGTCTTCGATGCTCCAAAGGACTTTAGGTTT  960
D  R  F  V  E  D  G  K  K  K  T  T  F  F  K  G  G  K  K  L
GATCGCTTCGTAGAAGATGGTAAGAAGAAAACAACGTTTTTCAAAGGAGGAAAAAAGCTG 1020
K  S  Y  I  I  P  F  G  L  G  T  S  K  C  P  G  R  Y  F  A
AAGAGTTACATTATACCATTTGGACTTGGAACAAGCAAATGTCCAGGCAGATACTTTGCA 1080
I  N  E  M  K  L  L  V  I  I  L  L  T  Y  F  D  L  E  V  I
ATTAATGAAATGAAGCTACTAGTGATTATACTTTTAACTTATTTTGATTTAGAAGTCATT 1140
D  T  K  P  I  G  L  N  H  S  R  M  F  L  G  I  Q  H  P  D
GACACTAAGCCTATAGGACTAAACCACAGTCGCATGTTTCTGGGCATTCAGCATCCAGAC 1200
S  D  I  S  F  R  Y  K  A  K  S  W  R  S  ***
TCTGACATCTCATTTAGGTACAAGGCAAAATCTTGGAGATCCTGAAAGGGTGGCAGAGAA 1260

GCTTAGCGGAATAAGGCTGCACATGCTGAGCTCTGTGATTTGCTGTACTCCCCAAATGCA 1320

GCCACTATTCTTGTTTGTTAGAAAATGGCAAATTTTTATTTGATTGCGATCCATCCAGTT 1380

TGTTTTGGGTCACAAAACCTGTCATAAAATAAAGCGCTGTCATGGTGTaaaaaaatgtca 1440

tggcaatcatttcaggataaggtaaaataacgttttcaagtttgtacttactatgatttt 1500

tatcatttgtagtgaatgtgcttttccagtaataaatttgcgccagggtgattttttta 1560

attactgaaatcctctaatatcggttttatgtgctgccagaaaagtgtgccatcaatgga 1620

cagtataacaatttccagttttccagagaagggagaaattaagccccatgagttacgctg 1680

tataaaattgttctcttcaactataatatcaataatgtctatatcaccaggttacctttg 1740

cattaaatcgagttttgcaaaag 1763
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FIG. 1. **Sequence of partial rat *hct-1* cDNA and the encoded polypeptide.** The nucleotide sequence and translation product of the 1.4-kb cDNA clone 12 including additional sequence data derived from clone 7 (*lower case*). The two putative polyadenylation signals are *underlined*.

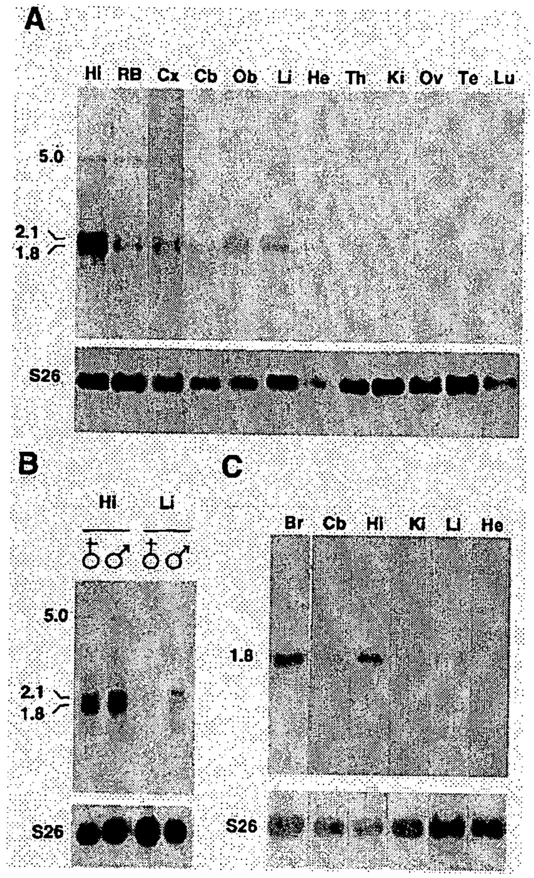
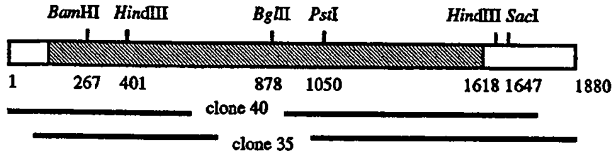


FIG. 2. **Northern analysis of *hct-1* expression in adult rat and mouse brain.** *A*, expression in rat brain and other tissues; *B*, sexually dimorphic expression in rat liver; *C*, expression in mouse tissues. Poly(A)$^+$ (*A*) or total (*B* and *C*) RNA from organs of adult animals was resolved by gel electrophoresis; the hybridization probe was rat *hct-1* cDNA clone 12 (1.4 kb); the probe for the loading control (below) corresponds to ribosomal protein S26. Tissues analyzed were: *Hi*, hippocampus; *RB*, remainder of brain lacking hippocampus; *Cx*, cortex; *Cb*, cerebellum; *Ob*, olfactory bulb; *Li*, liver; *He*, heart; *Th*, thymus; *Ki*, kidney; *Ov*, ovary; *Te*, testis; *Lu*, lung.

kb) was used to investigate the expression of *hct-1* mRNA in rat brain and other organs. While preliminary *in situ* hybridization experiments did not permit unambiguous localization of *hct-1* transcripts, we confirmed expression in the hippocampus, predominantly in the cell layers of the dentate gyrus, with weaker expression in other hippocampal and brain regions (not presented). Upon Northern analysis (Fig. 2*A*) the *hct-1* probe identified three transcripts in hippocampus of 5.0, 2.1, and 1.8 kb, with the two smaller transcripts being particularly enriched in hippocampus. The larger transcript was only detectable in brain, while the two smaller transcripts were also present in liver (and, at much lower levels, in kidney) but were not detected in other organs tested including adrenal (not shown), testis, and ovary. In brain, expression was also detected in olfactory bulb and cortex while very low levels were present in cerebellum (Fig. 2*A*). Whereas the 1.8- and 2.1-kb transcripts are thought to derive from alternative utilization of polyadenylation sites within *hct-1* transcripts, we were unable to confirm that the 5.0-kb transcript is encoded by the same gene because we failed to isolate correspondingly large cDNAs from the rat brain library and an equivalent 5-kb transcript was not detected in mouse (see below).

Sexual Dimorphism of hct-1 Expression in Liver but Not in Brain—The expression of several CYPs is known to be sexually dimorphic in liver (reviewed by Morgan and Gustafsson (39)). We therefore inspected liver and brain of male and female rats for the presence of *hct-1* transcripts. In Fig. 2*B* the *hct-1* probe revealed the 1.8-, 2.1-, and 5.0-kb transcripts in both male and female brain, with the 2.1-kb *hct-1* transcript predominating. While levels of *hct-1* mRNAs in liver were reduced greater than 20-fold over those detected in brain, *hct-1* transcripts were only significant in liver from male animals; expression in females was barely detectable, demonstrating that hepatic expression of *hct-1* is sexually dimorphic. The 5.0-kb transcript was not detected in liver.

Isolation of Mouse hct-1 cDNA Clones—Because the *hct-1* transcripts identified (predominantly 1.8 and 2.1 kb) are longer than the largest cDNA clone (1.4 kb) obtained from our rat hippocampus library we pursued studies with the mouse *hct-1* ortholog. A mouse liver cDNA library was screened using a rat *hct-1* probe, and four clones were selected, none containing a poly(A) tail. Two (clones 33 and 35, both 1.8 kb) gave identical DNA sequences at both their 5′ and 3′ ends, and this sequence was approximately 91% similar to rat *hct-1* (not shown). The remaining two clones, 23 and 40, were also identical to each other and were related to the other clones except for a 5′ extension (59 nt) and a 3′ deletion (99 nt). The complete DNA

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ggcaggcacagcctctggtctaagaagagagggcactgtgcagaagccatcgctccctaC     60
                                    M  Q  G  A  T  T  L  D  A  A  S  P  G  P   14
AGAGCCGCCAGCTCGTCGGGATGCAGGGAGCCACGACCCTAGATGCCGCCTCGCCAGGGC    120
 L  A  L  L  G  L  L  F  A  A  T  L  L  L  S  A  L  F  L  L   34
CTCTCGCCCTCCTAGGCCTTCTCTTTGCCGCCACCTTACTGCTCTCGGCCCTGTTCCTCC    180
 T  R  R  T  R  R  P  R  E  P  P  L  I  K  G  W  L  P  Y  L   54
TCACCCGGCGCACCAGGCGCCCTCGTGAACCACCCTTGATAAAAGGTTGGCTTCCTTATC    240
 G  M  A  L  K  F  F  K  D  P  L  T  F  L  K  T  L  Q  R  Q   74
TTGGCATGGCCCTGAAATTCTTTAAGGATCCGTTAACTTTCTTGAAAACTCTTCAAAGGC    300
 H  G  D  T  F  T  V  F  L  V  G  K  Y  I  T  F  V  L  N  P   94
AACATGGTGACACTTTCACTGTCTTCCTTGTGGGGAAGTATATAACATTTGTTCTGAACC    360
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CTTTCCAGTACCAGTATGTAACGAAAAACCCAAAACAATTAAGCTTTCAGAAGTTCAGCA    420
 R  L  S  A  K  A  F  S  V  K  K  L  L  T  D  D  D  L  N  E  134
GCCGATTATCAGCGAAAGCCTTCTCTGTAAAGAAGCTGCTTACTGATGACGACCTTAATG    480
 D  V  H  R  A  Y  L  L  L  Q  G  K  P  L  D  A  L  L  E  T  154
AAGACGTTCACAGAGCCTATCTACTTCTACAAGGCAAACCTTTGGATGCTCTTCTGGAAA    540
 M  I  Q  E  V  K  E  L  F  E  S  Q  L  L  K  I  T  D  W  N  174
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 T  E  R  I  F  A  F  C  G  S  L  V  F  E  I  T  F  A  T  L  194
ACACAGAAAGAATATTTGCATTCTGTGGCTCACTGGTATTTGAGATCACATTTGCGACTC    660
 Y  G  K  I  L  A  G  N  K  K  Q  I  I  S  E  L  R  D  D  F  214
TATATGGAAAAATTCTTGCTGGTAACAAGAAACAAATTATCAGTGAGCTAAGGGATGATT    720
 F  K  F  D  D  M  F  P  Y  L  V  S  D  I  P  I  Q  L  L  R  234
TTTTTAAATTTGATGACATGTTCCCATACTTAGTATCTGACATACCTATTCAGCTTCTAA    780
 N  E  E  S  M  Q  K  K  I  I  K  C  L  T  S  E  K  V  A  Q  254
GAAATGAAGAATCTATGCAGAAGAAAATTATAAAATGCCTCACATCAGAAAAAGTAGCTC    840
 M  Q  G  Q  S  K  I  V  Q  E  S  Q  D  L  L  K  R  Y  Y  R  274
AGATGCAAGGACAGTCAAAAATTGTTCAGGAAAGCCAAGATCTGCTGAAAAGATACTATA    900
 H  D  D  P  E  I  G  A  H  H  L  G  F  L  W  A  S  L  A  N  294
GGCATGACGATTCTGAAATAGGAGCACATCATCTTGGCTTTCTCTGGGCCTCTCTAGCAA    960
 T  I  P  A  M  F  W  A  M  Y  Y  I  L  R  H  P  E  A  M  E  314
ACACCATTCCAGCTATGTTCTGGGCAATGTATTATATTCTTCGGCATCCTGAAGCTATGG   1020
 A  L  R  D  E  I  D  S  F  L  Q  S  T  G  Q  K  K  G  P  G  334
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 I  S  V  H  F  T  R  E  Q  L  D  S  L  V  C  L  E  S  T  I  354
GAATTTCAGTCCACTTCACCAGAGAACAATTGGACAGCTTGGTCTGCCTGGAAAGCACTA   1140
 L  E  V  L  R  L  C  S  Y  S  S  I  I  R  E  V  Q  E  D  M  374
TTCTTGAGGTTCTGAGGCTGTGCTCATACTCCAGCATCATCCGAGAAGTGCAGGAGGATA   1200
 N  L  S  L  E  S  K  S  F  S  L  R  K  G  D  F  V  A  L  P  394
TGAATCTCAGCTTAGAGAGTAAGAGTTTCTCTCTGCGGAAAGGAGATTTTGTAGCCCTCT   1260
 P  P  L  I  H  N  D  P  E  I  P  D  A  P  K  E  F  R  F  D  414
TTCCTCCACTCATACACAATGACCCGGAAATCTTCGATGCTCCAAAGGAATTTAGGTTCG   1320
 R  F  I  E  D  G  K  K  K  S  T  F  F  K  G  G  K  R  L  K  434
ATCGGTTCATAGAAGATGGTAAGAAGAAAAGCACGTTTTTCAAAGGAGGGAAGAGGCTGA   1380
 T  Y  V  M  P  F  G  L  G  T  S  K  C  P  G  R  Y  F  A  V  454
AGACTTACGTTATGCCTTTTGGACTCGGAACAAGCAAATGTCCAGGGAGATATTTTGCAG   1440
 N  E  M  K  L  L  L  I  E  L  L  T  Y  F  D  L  E  I  I  D  474
TGAACGAAATGAAGCTACTGCTGATTGAGCTTTTAACTTATTTTGATTTAGAAATTATCG   1500
 R  K  P  I  G  L  N  H  S  R  M  F  L  G  I  Q  H  P  D  S  494
ACAGGAAGCCTATAGGGCTAAATCACAGTCGGATGTTTTTAGGTATTCAGCACCCCGATT   1560
 A  V  S  F  R  Y  K  A  K  S  W  R  S  ***                   507
CTGCCGTCTCCTTTAGGTACAAAGCAAAATCTTGGAGAAGCTGAAAGTGTGGCAGAGAAG   1620

CTTTGCAGAGTAAGGCTGCATGTGCTGAGCTCCGTGATTTGGTGCACTCCCCCAAATGCA   1680

ACCGCTACTCTTGTTTGAAAATGGCAAATTTATATTTGGTTGAGATCAATCCAGTTGGTT   1740

TTGGGTCACAAAACCTGTCATAAAATAAAGCAGTGTGATGGtttaaaaaatgtcatggca   1800

atcatttcaggataaggtaaaataacattttcaagtttgtacttactatgatttttatca   1860

tttgtagtgaatgtgctttt  1880
```

FIG. 3. **Mouse *hct-1* cDNA and the sequence of the encoded polypeptide.** The restriction map of the cDNA (*top*) corresponds to the compilation of two independent clones sequenced; the *cross-hatched box* indicates the coding region. The nucleotide sequence and translation product (*bottom*) are derived from this compilation. *Lower case* sequences indicate the 59 additional 5′ nucleotides in clone 40 and the 99 additional 3′ nucleotides in clone 35. The putative polyadenylation site is *underlined.*

sequences of clones 35 and 40 were therefore determined.

The sequences obtained were identical throughout the region of overlap. The mouse *hct-1* open reading frame commences with a methionine at nucleotide 81 (numbering from clone 40) and terminates with a TGA codon at nucleotide 1600, encoding a protein of 507 amino acids (Fig. 3). At the 5′ end the ATG initiation codon leading the open reading frame does not correspond to the translation initiation consensus sequence (YYAYYATGR (40)). However, the 5′-untranslated region cloned is devoid of other possible initiation codons, and an in-frame termination triplet (TAA) lies 20 codons upstream of the ATG. The encoded polypeptide sequence aligns well with other cytochrome P450 sequences (see below); we surmise that the ATG at position 81 represents the correct start site for translation. At the 3′ end the truncation of clone 40 lies entirely in the non-coding region downstream of the stop codon. Neither clone contained a poly(A) tail, but both contained a potential polyadenylation sequence (AATAAA) at a position corresponding precisely to that seen in the rat cDNA.

A

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MoHct-1   1 MQGATTLDAASPGPLALLGLLFAATLLLSALFLLTRRTRRPREPPLIKGW  50
HuCYP7    1 M--MTTSLIWGIAIAACCCL---------WLILGIRRRQTG-EPPLENGL  38

 51 GWLPYLGMALKFFKDPLTFLKTLQRQHGDTFTVFLVGKYITFVLNPFQYQYVTKNPKQLSFQKF 112
 39 GLIPYLGCALQFGANPLEFLRANQRKHGHVFTCKLMGKYVHFITNPLSYHKVLCHGKYFDWKKF 100

113 SSRLSAKAFSVKKLLT-DDDLNEDVHRAYL-LLQGKPLDALLETMI---QEVKELFESQLLKIT 171
101 HFATSAKAFGHRSIDPMDGNTTENINDTFIKTLQGHALNSLTESMMENLQRIMRPPVSSNSKTA 164

172 DWNTERIFAFCGSLVFEITFATLYGKILA---GNKKQIISELRDDFFKFDDM-FPYLVSDIPIQ 231
165 AWVTEGMYSFCYRVMFEAGYLTIFGRDLTRRDTQKAHILNNL--DNFKQFDKVFPALVAGLPIH 226

240 LLRNEESMQKKIIKCLTSEKVAQMQGQSKIVQESQDLLKRYYRHDDPEIGAHHLGFLWASLANT 295
227 MFRTAHNAREKLAESLRHENLQKRESISELISLRMFLNDTLSTFDDLEKAKTHLVVLWASQANT 290

296 IPAMFWAMYYILRHPEAMEALRDEIDSFLQSTGQKKG-PGISVHFTREQLDSLVCLESTILEVL 358
291 IPATFWSLFQMIRNPEAMKAATEEVKRTLENAGQKVSLEGNPICLSQAELNDLPVLNSIIKESL 354
                                                              C
359 RLCSYSSIIREVQEDMNLSLESKSFSLRKGDFVALPPPLIHNDPEIPDAPKEFRFDRF-IEDGK 421
355 RLSSASLNIRTAKEDFTLHLEDGSYNIRKDSIIALYPQLMHLDPEIYPDPLTFKYDRYLDENGK 418
                                    B
422 KKSTFFKGGKRLKTYVMPFGLGTSKCPGRYFAVNEMKLLLIELLTYFDLEIID--RKPIGLNHS 483
419 TKTTFYCNGLKLKYYYMPFGSGATICPGRLFAIHEIKQFLILMLSYFELELIEGQAKCPPLDQS 482

484 RMFLGIQHPDSAVSFRYKAKSWRS* 507
483 RAGLGILPPLNDIEFKYKFKHL* 504
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B

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MoHct-1  F G L G T S K C P G R Y F A
HuCYP7   F G S G A T I C P G R L F A
CYP17    F G A G P R S C V G E M L A
CYP11B   F G F G M R Q C L G R R L A
CYP21B   F G C G A R V C L G E S L A
CYP11A1  F G W G V R Q C L G R R I A
CYP27    F G Y G V R A C L G R R I A
```

C

```
V C L E S T I L E V L R L C S
P V L N S I I K E S L R L S S
V L L E A T I R E V L R I R P
P L L R A A L K E T L R L Y P
P L L N A T I A E V L R L R P
P L L K A S I K E T L R L H P
P L L K A V L K E T L R L Y P
```

FIG. 4. **Alignment of mouse *hct-1* with human CYP7 (cholesterol 7α-hydroxylase) and steroidogenic P450 s.** *A*, identical amino acids are indicated by a *bar*; *hyphens* in the amino acid sequences indicate gaps introduced during alignment. The N-terminal hydrophobic leader sequences are *underlined.* The position of the conserved Thr residue within the O_2-binding pocket of other CYPs (43), but replaced by Asn in *hct-1* (position 294) and CYP7, is indicated by an *asterisk*. *B* and *C*, conserved residues in the heme-binding (residues 440–453, *B*) and postulated steroidogenic (residues 348–362, *C*) domains conserved between *hct-1* and other similar CYPs (*overlined* in *A*). Sequences are human CYP7 (7α-hydroxylase (36)), bovine CYP17 (17α-hydroxylase (42)), human CYP11B1 (steroid β-hydroxylase (43)), bovine CYP21B (21-hydroxylase (11)), human CYP11A1 (P450scc; cholesterol side chain cleavage (44)), and rabbit CYP27 (27-hydroxylase (45)).

Structure of Mouse hct-1 Polypeptide—As anticipated, nucleotide sequence homology of mouse *hct-1* was highest with human CYP7 (~56% identity over the coding region). At the polypeptide level the mouse open reading frame shows 81% identity to the rat *hct-1* polypeptide over 414 amino acids; the overall degree of similarity may be different as the full protein sequence of rat *hct-1* is not known. Both the human (CYP7 (36)) and rat (37, 38) CYP7 polypeptides share 39% amino acid sequence identity to mouse *hct-1*. Fig. 4*A* presents the alignment of mouse *hct-1* polypeptide with human CYP7.

The N terminus of the *hct-1* polypeptide is hydrophobic, a

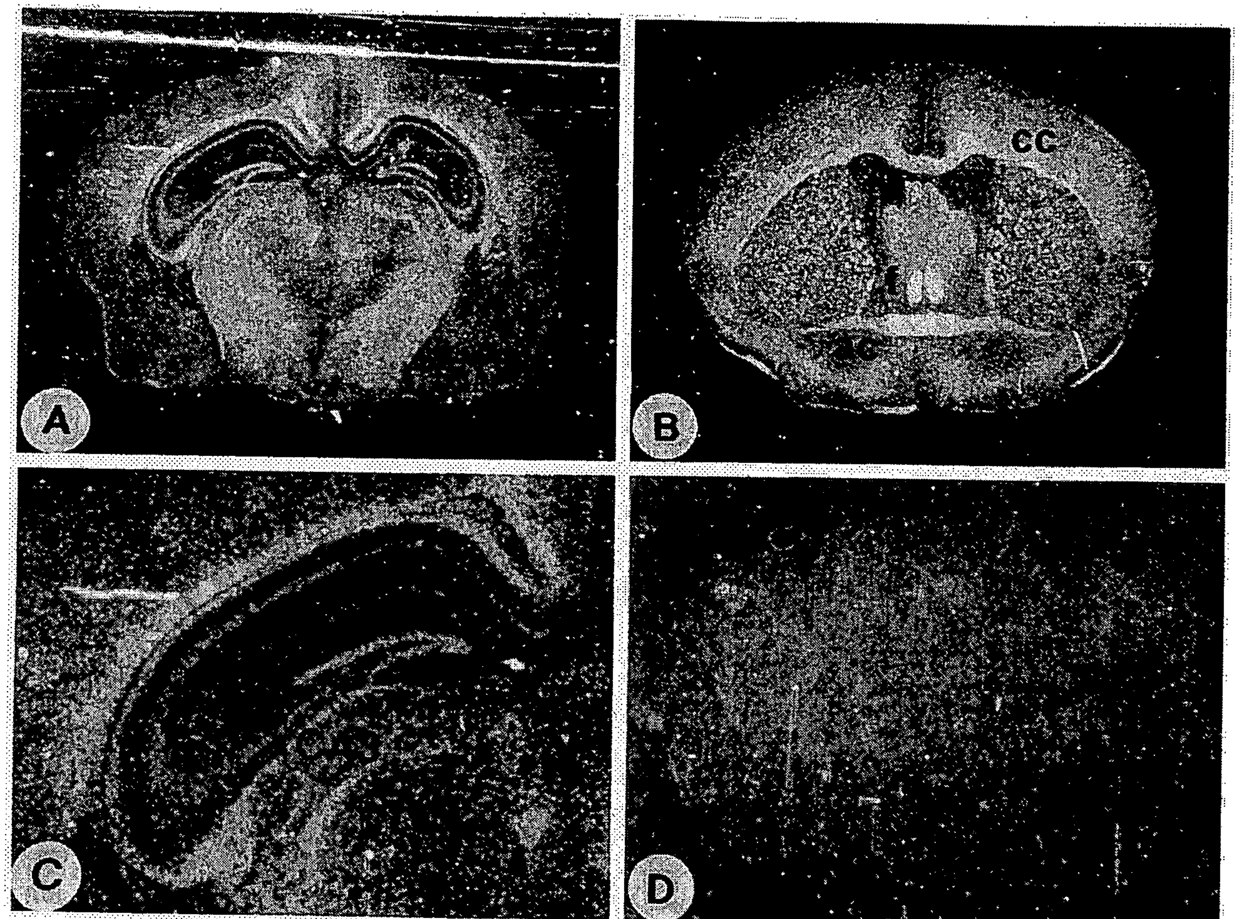

FIG. 5. **Analysis of *hct-1* expression in adult mouse brain.** The hybridization probe was a synthetic oligonucleotide corresponding to the 3′-untranslated region of mouse *hct-1* cDNA. *A*, coronal section; *B*, coronal section, rostral to *A*, showing hybridization in corpus callosum (*cc*), fornix (*f*), and anterior commissure (*ac*); *C*, enlargement of section through the hippocampus (*DG*, dentate gyrus); *D*, section adjacent to the section in *A* hybridized with an oligonucleotide specific for opsin (negative control).

feature shared by microsomal CYPs. This portion of the polypeptide is thought to insert into the membrane of the endoplasmic reticulum. Consistent with microsomal CYPs, the N terminus lacks basic amino acids prior to the hydrophobic core (amino acids 9–34). Previous alignment studies have highlighted conserved regions within CYP polypeptides (*e.g.* Ref. 46). CYPs contain a highly conserved motif, F*XX*G*XXX*C*X*G(*XXX*A), present in 202 of the 205 compiled sequences (1), thought to represent the heme binding site with the arrangement of amino acids around the cysteine residue postulated to preserve the three-dimensional structure of this region for ligand binding (41). This motif is fully conserved in *hct-1* (Fig. 4*B*). A second domain, which may be conserved in CYPs responsible for steroid interconversions (47, 36), also is featured in *hct-1* though an invariant Pro residue is replaced, in *hct-1*, by Val (Fig. 4*C*).

Expression Pattern of Mouse hct-1—To verify enriched expression of *hct-1* in hippocampus we performed Northern and *in situ* hybridization analyses on mouse material. In contrast to the situation in rat, the 1.4-kb clone 12 detected only a 1.8-kb transcript; the 2.1- and 5.0-kb transcripts appeared to be absent (Fig. 2*C*). This apparent absence may only reflect a lower abundance of longer transcripts because some mouse cDNA clones isolated clearly extend beyond the upstream polyadenylation site thought to generate the shorter (1.8 kb) transcript in rat.

To refine this analysis, a 42-mer oligonucleotide was designed according to the DNA sequence of the 3′-untranslated region of the cDNA clone upstream of the first polyadenylation site to minimize cross-hybridization with other CYP mRNAs. Coronal sections of mouse brain were hybridized, emulsion dipped, and exposed for autoradiography (Fig. 5). Transcripts were detected throughout mouse brain and were not restricted to the hippocampus (Fig. 5, *A* and *B*). Strongest expression was observed in the corpus callosum, the anterior commissure, and fornix while, as in rat, hippocampal expression was particularly prominent in the dentate gyrus (Fig. 5*C*). Moderate expression levels, comparable with those observed in hippocampus, were observed in cerebellum, cortex, and olfactory bulb.

A Single Gene for hct-1 in Mouse, Rat, and Human—Because CYPs comprise a family of related enzymes we determined whether close homologs of *hct-1* are present in the mammalian genome. The rat *hct-1* probe (1.4 kb) was used to probe a genomic Southern blot of rat, mouse, and human DNA (Fig. 6) giving a simple pattern of cross-hybridizing bands in all samples. In *Bam*HI-cut human DNA only a single major cross-hybridizing band (4 kb) was detected (Fig. 6), while reprobing with the 300-nt clone 14–5a again yielded, in each lane, a single cross-hybridizing band (not shown). These data argue that a single conserved and unduplicated *hct-1* gene is present in mouse, rat, and human. However, we cannot rigorously exclude the possibility that the mammalian genome might contain close homologs of *hct-1* that would be only poorly detected by cross-hybridization (<70–80% homology).

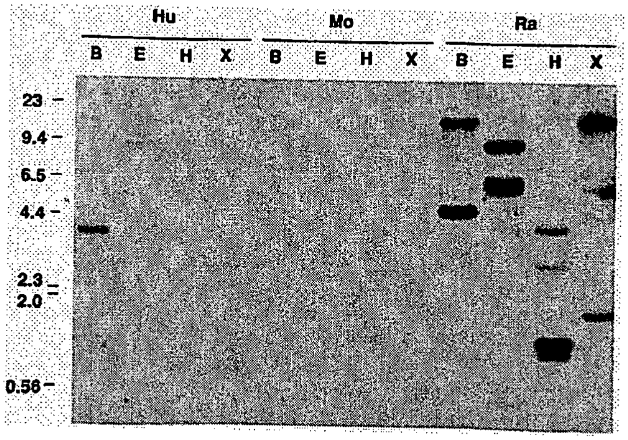

FIG. 6. **Southern analysis of *hct-1* coding sequences in mouse (*Mo*), rat (*Ra*), and human (*Hu*).** Total DNA was cleaved as indicated with restriction endonucleases (*B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII; *X*, *Xba*I), resolved by agarose gel electrophoresis, and probed with rat *hct-1* cDNA clone 12 before exposure to autoradiography.

DISCUSSION

To characterize transcripts enriched in the hippocampal formation we isolated clones corresponding to *hct-1* from a rat hippocampus cDNA library. In rat, expression appeared to be most abundant in hippocampus with some expression in cortex and substantially less expression in other brain regions. Elsewhere in the body transcripts were only detected in liver and, to a lesser extent, in kidney; expression was barely detectable in ovary, testis, and adrenal, also sites of steroid transformations and CYP activity. Hepatic expression was sexually dimorphic with *hct-1* mRNA barely detectable in female liver. In rat brain and liver, two *hct-1* transcripts of 1.8 and 2.1 kb appear to be generated by alternative polyadenylation; a 5.0-kb transcript was weakly detected in rat brain but was not observed in mouse.

Sequence analysis of *hct-1* cDNA clones revealed that *hct-1* encodes a novel cytochrome P450 (CYP). Although the mouse cDNA coding region appears complete, the absence of a consensus translation initiation site (40) flanking the presumed initiation codon could indicate that *hct-1* polypeptide synthesis is subject to regulation at the level of translation initiation. Homology was highest with rat and human cholesterol 7α-hydroxylase (36–38), known as CYP7. While related, *hct-1* is distinct from CYP7, sharing only 39% homology over the full length of the protein. CYP polypeptides sharing greater than 40% sequence identity are generally regarded as belonging to the same family (1); *hct-1* and CYP7 (39% similarity) are borderline. The conservation of other unique features between *hct-1* and CYP7 (see below), however, argues for a close relationship, and *hct-1* has been designated "CYP7B" (Cyp7b in mouse) by the Committee on Standardized Cytochrome P450 Nomenclature.[3]

From the *hct-1* leader sequence we surmise that the *hct-1* polypeptide resides, like CYP7, in the endoplasmic reticulum and not in mitochondria, the other principal cellular site of CYP activity. The strictly conserved heme binding site motif F*XX*G*XXX*C*X*G(*XXX*A) (1) is present in *hct-1* (residues 440–453) while the postulated "steroidogenic domain" (*e.g.* Ref. 36) shared by CYPs responsible for steroid interconversions is also present in *hct-1* (amino acids 348–362), except that a consensus Pro residue is replaced by Val in both the mouse and rat *hct-1* polypeptides. Of 34 CYP sequences compiled by Nelson and Strobel (46), only 4 contain an amino acid residue other than Pro at this position. Whereas 2 of these harbor an unrelated amino acid (Glu; CYP3A1, CYP3A3), interestingly, a Val residue is present in bovine CYP17 (steroid 17α-hydroxylase (42)) at a position equivalent to that in *hct-1* while human CYP17 harbors a conservative substitution at this site (Leu (47)). Despite this similarity, however, the overall extent of homology between *hct-1* and CYP17 (22.5%, not shown) is lower than with CYP7 (39%).

Neither *hct-1* nor CYP7 appears to contain a conserved O_2 binding pocket (equivalent to residues 285–301 in *hct-1*) as highlighted by Poulos (41). Crystallographic studies on the bacterial CYP101 indicated that a Thr residue (corresponding to position 294 in *hct-1*) disrupts helix formation in that region and is important in providing a structural pocket for an oxygen molecule (41). Site-directed mutagenesis of this Thr residue in both CYP4A1 and CYP2C11 demonstrated that this region can influence substrate specificity and affinity (48). In both *hct-1* and CYP7 the conserved Thr residue is replaced by Asn. This modification suggests that *hct-1* and CYP7 are both structurally distinct from other CYPs in this region; this may be reflected both in modified oxygen interaction and substrate choice.

The sexual dimorphism of *hct-1* expression observed in rat resembles that observed with a number of other CYPs. CYP2C12 is expressed preferentially in liver of the female rat while, like *hct-1*, CYP2C11 is highly expressed in male liver but only at low levels in the female tissue (49). This dimorphic expression pattern of CYP2C family members is thought to be determined by the dimorphism of pulsatility of growth hormone secretion (39). Brain expression of *hct-1* is not subject to this control, suggesting that regulatory elements determining *hct-1* expression in brain differ from those utilized in liver. However, we have not examined species other than rat; it cannot be assumed that the same regulation will exist in other species. Indeed, sexually dimorphic gene expression is not necessarily conserved between different strains of mouse (*e.g.* Ref. 50).

Expression of *hct-1* was widespread in mouse brain. Here the expression pattern was most consistent with glial expression, but further experiments will be required to compare neuronal and non-neuronal levels of expression. In mouse brain only the 1.8-kb transcript was detected. However, cDNAs were obtained corresponding to transcripts extending beyond the first polyadenylation site; such extended transcripts are thought to give rise to the 2.1-kb transcript in rat. This suggests the downstream polyadenylation site seen in rat *hct-1* is underutilized in mouse *hct-1* or absent. While *in situ* hybridization studies of *hct-1* in rat brain were inconclusive, a difference in expression pattern between mouse and rat appears likely; further work will be required to confirm this. Such a difference would be unsurprising because cytochromes P450 are well known to vary widely in their level and pattern of expression in different species; for instance, hepatic testosterone 16-hydroxylation levels differ by more than 100-fold between guinea pig and rat (51).

Our data indicate that the *hct-1* gene is present in rat, mouse, and human and does not appear to be duplicated in the mammalian genome. While CYP genes are scattered over the mouse and human genomes, CYP subfamilies can cluster on the same chromosome. For instance, the human CYP2A and -2B subfamily genes are linked to chromosome 19, CYP2C and -2E subfamilies are located on human chromosome 10, and the mouse Cyp2a, -2b, and -2e subfamilies are present on mouse chromosome 7 (reviewed by Paine (52)). The gene encoding human CYP7 is located on chromosome 8q11-q12 (53). It will be of future interest to determine the chromosomal location of the

[3] D. R. Nelson, personal communication.

human *hct-1* homolog. Experiments are in progress to address this question.

What role might *hct-1* play in the brain? In the adult CYPs are generally expressed abundantly in liver, adrenal, and gonads, while the level of CYP activity in brain is estimated to be 0.3–3% of that found in liver (see Ref. 54). Unusually, levels of *hct-1* mRNA expression in rat and mouse brain far exceed those in liver, and it could be argued that the primary function of *hct-1* lies in the central nervous system. Our data argue that *hct-1* is related to the steroid-metabolizing CYPs, and most similar to CYP7, this may suggest that the substrate for *hct-1*, so far unknown, is likely to be related to cholesterol or one of its steroid metabolites. This interpretation is borne out by the presence, in *hct-1*, of a postulated steroidogenic domain (37) that appears to be conserved in steroid-metabolizing CYPs. However, the functional significance of this domain in steroidogenic CYPs has not yet been demonstrated; it remains a possibility that *hct-1* metabolizes substrates other than cholesterol or steroids. While experiments are presently under way to determine the substrate specificity of *hct-1*, the possibility that *hct-1* might act on cholesterol or its steroid metabolites in brain is of some interest. CYP7 (cholesterol 7α-hydroxylase) is responsible for the first step in the metabolic degradation of cholesterol. This is of note in view of the association of particular alleles of the *APOE* gene encoding the cholesterol transporter protein apolipoprotein E with the onset of Alzheimer's disease (55, 56), a neurodegenerative condition whose cognitive impairments are associated with early dysfunction of the hippocampus. The documented ability of cholesterol-derived steroids to interact with neurotransmitter receptors and modulate both synaptic plasticity and cognitive function (12–19) may suggest that *hct-1* and its metabolic product(s) regulate neuronal function *in vivo*.

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